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(54) Title: NUTRITIONAL FORMULAS SUPPLEMENTED WITH NUCLEOTIDES, AND PROCESSES THEREOF

(57) Abstract

Milk-based or non-milk-based nutritional formulas are supplemented with nucleotides so that the total levels of free bioavailable nucleotides in the supplemented formulas match the corresponding levels of free bioavailable nucleotides present in human milk. The amounts of nucleotides are adjusted depending on the background levels of free bioavailable nucleotides present in the other formula ingredients. The nucleotides are added after heat processing but before homogenization. The addition of nucleotides to infant formulas at these levels to substantially match the levels of free bioavailable nucleotides to the levels found in human milk results in a stool consistency of formula-fed infant closer to that of human milk-fed infants.

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# NUTRITIONAL FORMULAS SUPPLEMENTED WITH NUCLEOTIDES, AND PROCESSES THEREOF

## FIELD OF THE INVENTION

The present invention relates to nutritional formulas containing nucleotides and/or nucleosides. Specifically, the present invention relates to milk-based and non-milk-based nutritional formulas which are supplemented with nucleotides and/or nucleosides and to processes for making such formulas. The present invention also relates to a method of reducing the incidence of constipation in infant-formula fed infants and to a method for softening stools of infant-formula fed babies.

## BACKGROUND OF THE INVENTION

Nucleotides are low molecular-weight compounds consisting of a nitrogenous base, a sugar moiety and one to three phosphate groups. They are essential in energy metabolism and enzymatic reactions and are the monomeric units of polymeric RNA and DNA. Nucleotides are an integral part of carbohydrate, lipid, protein and nucleic acid metabolism. Nucleosides are similar to nucleotides except that they do not include a phosphate group or groups. As used herein the term "nucleotide equivalents" shall mean either or both nucleotides and nucleosides.

Human milk contains a number of nucleotides and nucleosides. A number of studies identified nucleotides and nucleosides present in human milk and their levels. Nucleosides are believed to be present primarily in free bioavailable form. Nucleotides can be present in free bioavailable forms or in bound forms that have to be broken down in order to be metabolized. A number of studies have been carried out to determine nucleotides and nucleosides present in human milk and their levels in human milk. Examples of published results of such studies include the following: Janas, L.M. and Picciano, M.F., (1982), "The Nucleotide Profile of Human Milk," *Pediatr. Res.*, 16:659-62; Gil, A. and Sanchez-Medina, F., (1982), "Acid-Soluble Nucleotides in Human Milk at Different Stages of Lactation," *J. Dairy Res.*, 49:301-07; Leach, J.L., Baxter, J.H.,

Molitor, B.E., Ramstack, M.B., and Masor, M.L., (1995), "Total Potentially Available Nucleosides in Human Milk By Stage of Lactation," *Am. J. Clin. Nutr.*, 61:1224-30.

Cow's milk and nutritional formulas based on milk or non-milk ingredients generally also contain nucleotides and nucleosides. However, the types and/or levels of nucleotides and nucleosides present in human milk are different from those present in cow's milk and in nutritional formulas made from animal milk or made from non-milk ingredients.

Prior art discloses supplementing cow's milk, milk products and nutritional formulas with various nucleotides to make cow's milk, milk products or nutritional formulas similar to human milk. However, prior art is in disagreement as to what nucleotides should be added and at what concentrations to make the non-human milk, milk-based or non-milk based formulas similar to human milk. For example, U.S. Pat. No. 3,231,385 (Ziro et al.) discloses that there is a difference between nucleotides and nucleotide levels between human milk and cow's milk. The Ziro et al. patent discloses that to make the nucleotide content of cow's milk or other cow milk products similar to human milk, nucleotides which are present in human milk but substantially absent in cow's milk may be added. Specifically, the Ziro et al. patent discloses that "GMP, UMP, CMP and other nucleotide fractions may be added to cow's milk so as to make their content similar to that of human milk." Column 4, lines 70-72. The nucleotide fractions can be added "as such" or in the form of an alkali salt. See column 4, lines 17-22. The Ziro patent discloses specific levels of added nucleotides per liter of cow's milk without regard to background levels of these nucleotides.

Similarly, U.S. Pat. No. 5,066,500 discloses adding nucleotides and/or nucleosides to nutritional products and to formulas and processes for their preparation. The purpose of this addition is to provide formulas having enhanced physiological properties and also closely resemble human milk. The '500 patent recognizes that "it would be desirable that infant milk formulas have most of the substances present in human milk so as to produce the same physiological effects as human milk." Column 2, lines 2-5. Adenosine, guanosine, cytidine, inosine, uridine are the specific nucleosides disclosed in the '500 patent. Mono, di, and/or tri phosphates of these nucleosides or their

sugar derivatives are the nucleotides disclosed in the '500 patent. The '500 patent discloses adding at least one of these nucleotides or nucleosides. The '500 patent discloses adding from 1 to 300 mg of the nucleotides and/or nucleosides per 100 g of a diet formulation, preferably about 50 to about 250 mg and optionally 150 mg per 100 g of the formulation. The concentrations of these nucleotides in a liquid formulation from about 20 to about 600 mg per liter of the formulation, preferably about 100 to about 500 mg per liter. The optimum concentration is 300 mg per liter of the formulation. The disclosed amounts of nucleotides added per 100g of a non-milk infant formula are in the following ranges:

| | |
|---|---|
| Uridine or uridine phosphate | 1.86 - 17.40 mg |
| Guanosine or guanosine phosphate | 0.27 - 3.32 mg |
| Adenosine or adenosine phosphate | 4.25 - 9.5 mg |
| Cytidine or cytidine phosphate | 3.52 - 10.16 mg |
| Inosine or inosine phosphate | 0.0 - 1.92 mg |

The process disclosed in the '500 patent requires a homogenizing step before the addition of nucleotides.

U.S. Patents Nos. 5,488,039 and 5,492,899 (collectively referred as the Masor patents) rejected the approach of trying to match the levels of nucleotides in cow's milk to the levels found in human milk. Instead, the Masor et al. patents allegedly arrived at the disclosed levels of nucleotides based on performance of the formula. In other words, the levels called for by the patents allegedly achieve similar clinical results as those present in human milk. The Masor et al. patents suggest that the pertinent measure of nucleotides is total potentially available nucleotide (TPAN) levels which include both free and bound nucleotides. The Masor et al. patents disclose addition of very specific levels of four nucleotides (CMP, UMP, AMP, and GMP) to the formula after the heat processing and homogenization stops. The specific levels of total potentially available nucleotides disclosed in the Masor patents are as follows:

| Nucleotide | TPAN Concentration (mg/L) |
|---|---|
| CMP | 29-39 |
| UMP | 15-21 |
| AMP | 10-16 |
| GMP | 14-20 |

The concentrations of free bioavailable nucleotides used to supplement infant formulas as disclosed in the Masor et al. patents are higher than the corresponding concentrations found in human milk. Since the roles and functions of nucleotides are not fully understood, these high levels of nucleotides present a risk of adverse effects on the babies fed with the supplemented formulas made in accordance with the Masor et al. patents.

Some of the adverse effects associated with the consumption of excessive nucleotides are taught in the publication of A. Griebsch and N. Zöller, "Effect of ribomonucleotides given orally on uric acid production in man," *Adv. Exp. Med. Biol.*, (1974), Vol. 41B, pp. 443-49. Indeed, the current European Commission Directive prevents the sale in European Community countries of infant formulas having added nucleotide at levels above those specified in the directive. Specifically, the directive provides that the total concentration of nucleotides shall not exceed 5 mg/100 kcal. and sets forth the following maximum levels for added nucleotides:

| Nucleotide | Maximum Level |
|---|---|
| Cyidine 5' - monophosphate | 2.50 mg/100 kcal |
| Uridine 5' - monophosphate | 1.75 mg/100 kcal |
| Adenosine 5' - monophosphate | 1.50 mg/100 kcal |
| Guanosine 5' - monophosphate | .50 mg/100 kcal |
| Inosine 5' - monophosphate | 1.00 mg/100 kcal |

In addition to adding nucleotides for nutritional purposes, nucleotides have also been added for therapeutic purposes. Therapeutic uses of nucleotides are disclosed in several patents. For example, U.S. Pat. No. 4,544,559 discloses the addition of several specific nucleotides to cow's milk in a specific molar ratio in order to enhance the growth of the bifido bacteria B. bifidum Ti. The '559 patent discloses that all five specified nucleotides needed to be present and in specified molar ratios to achieve the desired results. The five nucleotides include: uridine monophosphate (UMP), guanosine

monophosphate (GMP), adenosine monophosphate (AMP), cytidine monophosphate (CMP) and inosine nucleotides (IMP). The specified ratios for AMP, CMP, GMP, UMP and IMP were 1:1:1:3:0.5, respectively. U.S. Pat. No. 4,994,442 discloses adding of a "therapeutically effective amount" of the ingredients, including nucleotides or nucleosides to a formula. The formula containing nucleotides or nucleosides is intended for patients in need of enhanced immune response of T-cells and for the stimulation or repair and regeneration of intestinal gut cells.

Thus, prior art discloses that cow's milk, milk products and milk and non-milk-nutritional formulas can be supplemented with nucleotides to make them more similar to human milk or for certain therapeutic purposes. However, there is a disagreement as to the type and levels of nucleotides and/or nucleosides that should be added and what criteria to use for selecting the levels of nucleotides.

There is, therefore, an unsatisfied need for an approach for supplementing milk and non-milk nutritional formulas with nucleotides so as to match the function of nucleotides present in human milk. The present invention addresses and satisfies this need.

## SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, milk-based or non-milk-based nutritional formulas are supplemented with free bioavailable nucleotide equivalents so that the resulting levels of free bioavailable nucleotide equivalents in the formulas substantially match the general levels of the corresponding nucleotides present in human milk. The levels of total free bioavailable nucleotides in the nutritional formulas of the present invention are generally in the following approximate ranges:

| Nucleotide | Free Nucleotides (mg/L) |
|---|---|
| CMP | 8-15 |
| UMP | 5-11 |
| GMP | 2-6 |
| AMP | 3-7 |

Since other ingredients present in the formulas contain background levels of free bioavailable nucleotides, the levels of nucleotides that are added have to be adjusted

based on the background to achieve the above listed levels of free bioavailable nucleotides.

In accordance with another aspect of the present invention the levels of total free bioavailable nucleotides in the formulas of the present invention are in the following ranges:

| Nucleotide | Free Nucleotides (mg/L) |
|---|---|
| CMP | 10-11 |
| UMP | 5-7 |
| GMP | 3-5 |
| AMP | 4-6 |

In accordance with yet another aspect of the present invention, a dry powder nutritional formula contains the following nucleotides per gram of the powder formula:

(1) from about 0.039 mg to about 0.086 mg UMP;

(2) from about 0.016 mg to about 0.047 mg GMP;

(3) from about 0.023 mg to about 0.055 mg AMP;

(4) from about 0.063 mg to about 0.117 mg CMP.

In accordance with a further aspect of the present invention, the nucleotides at levels that match the levels found in human milk are added to a liquid formula after the heat treatment but before homogenization.

In accordance with still another aspect of the present invention, a method of reducing incidence of constipation in formula fed infants comprises feeding infants the above-described infant formulas with free bioavailable nucleotides at levels substantively matching the levels of free bioavailable nucleotides in human milk.

In accordance with a still further aspect of the present invention, a process for preparing a supplemented infant nutritional formula comprises the steps of measuring the amount, if any, of free bioavailable nucleotides present in the formula and adding free bioavailable nucleotides to the formula such that nucleotides present in the resulting supplemented formula substantially match the mean value of free bioavailable nucleotides present in human milk (the mean value of human milk as defined in J.L.

Leach, et al. (1995) "Total Potentially Available Nucleosides in Human Milk by Stage of Lactation," *Am. J. Clin. Nutri.*, 61:1224-30, hereby incorporated by reference).

## DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that the beneficial effects of nucleotides present in human milk can best be achieved in nutritional formulas by matching the total levels of free bioavailable nucleotides in the formula to those of the corresponding free nucleotides present in human milk. Since standard ingredients of nutritional formulas generally contain small amounts of free bioavailable nucleotides (background levels), the amounts of free bioavailable nucleotides that are added must be adjusted to account for these background levels. The levels of free bioavailable nucleotide equivalents present in the nutritional formulas should be generally in the following ranges:

| Nucleotide | Free Nucleotides (mg/L) |
|---|---|
| CMP | 8-15 |
| UMP | 5-11 |
| GMP | 2-6 |
| AMP | 3-7 |

Preferably, the nucleotides are in the following ranges:

| Nucleotide | Concentration mg/L |
|---|---|
| AMP | 4-6 |
| GMP | 3-5 |
| CMP | 10-11 |
| UMP | 5-7 |

Excellent results were obtained at the following levels of free bioavailable nucleotide equivalents in a nutritional formula:

| Nucleotide | Concentration mg/L |
|---|---|
| AMP | 5 |
| GMP | 4 |
| CMP | 11 |
| UMP | 6 |

As used herein, the term "free bioavailable nucleotide equivalent" means and encompasses any and all of monomeric nucleotides, nucleosides and their salts. This term does not encompass polymeric forms of nucleotides or nucleosides and specifically it does not encompass nucleotide adducts or nucleotides present in DNA or RNA.

As used herein, the term "non-human protein" means animal proteins (such as, cow-, bovine- or sheep-milk proteins) or vegetable proteins (such as, soy rice or the like proteins) or proteins derived from animal or vegetable proteins.

Additionally, as used in connection with the description of the present invention, the terms CMP, AMP, GMP and UMP are not limited to monophosphate forms of nucleotides. Instead, these terms include the corresponding nucleosides and related free bioavailable nucleotide equivalents, including diphosphates (CDP, ADP, GDP, UDP) and triphosphates (CTP, ATP, GTP, UTP). For example, unless otherwise stated, the term CMP encompasses not only cytidine monophosphate but all monomeric cytidine nucleotides, nucleosides and salts of these nucleotides and nucleosides.

It is preferred that the nutritional formula supplemented with the nucleotides in accordance with the present invention be nutritionally complete. As used herein the term "nutritionally complete" means that the composition contains adequate nutrients to sustain healthy human life for extended periods. The nutritionally complete formula that can be supplemented with nucleotides in accordance with the present invention contains ingredients which are designed to meet the nutritional needs of the human infant namely, a protein, carbohydrate and lipid source and other nutrients such as vitamins and minerals. The amount of protein per 100 Cal. of total formula is typically about 1.8 g to about 4.5 g; the amount of lipid source per 100 Cal. of total formula is typically about 3.3 g to about 6 g; and the amount of carbohydrate source per 100 Cal. of total formula is typically about 7 g to about 14 g. The protein source can be non-fat milk solids, a combination of non-fat milk solids and whey protein, a hydrolysate of non-fat milk and/or why solids, soy protein isolates, or hydrolyzed soy protein isolates. The infant formula can be casein predominant or whey predominant. The carbohydrate source in the infant formula (other than starch) can be any carbohydrate known in the art to be suitable for use in infant formulas. Typical carbohydrate sources include sucrose, fructose, glucose,

maltodextrin, lactose, corn syrup, corn syrup solids, and the like. The formula may optionally contain starch, such as, nutritionally complete waxy corn, waxy rice and potato starch preferably in an amount effective to ameloriate regurgitation. Such an amount is typically about 1.8 to about 5 g of starch per 100 Cal. of formula; preferred is about 2 to about 4.7 g of starch per 100 Cal. of formula; more preferred is about 2 to about 3 g of starch per 100 Calories of formula. The lipid source in the infant formula can be any lipid or fat known in the art to be suitable for use in infant formulas. Typical lipid sources include milk fat, safflower oil, egg yolk lipid, olive oil, coconut oil, palm oil, palm kernel oil, soybean oil, sunflower oil, fish oil and fractions derived thereof such as palm olein, medium chain triglycerides (MCT), and esters of fatty acids wherein the fatty acids are, for example, arachidonic acid, linoleic acid, palmitic acid, stearic acid, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, oleic acid, lauric acid, capric acid, caprylic acid, caproic acid, and the like. High oleic forms of various oils are also contemplated to be useful herein such as high oleic sunflower oil and high oleic safflower oil. Medium chain triglycerides contain higher concentrations of caprylic and capric acid than typically found in conventional oils, e.g. approximately three-fourths of the total fatty acid content is caprylic acid and one-fourth is capric acid.

Nutritionally complete formulas contain all vitamins and minerals understood to be essential in the daily diet and these should be present in nutritionally significant amounts. Those skilled in the art appreciate that minimum requirements have been established for certain vitamins and minerals that are known to be necessary for normal physiological function. Those skilled in the art also understand that appropriate additional amounts (overages) of vitamin and mineral ingredients need to be provided to compensate for some loss during processing and storage of such compositions. To select a specific vitamin or mineral compound to be used in the infant formula of the invention requires consideration of that compound's chemical nature regarding compatibility with the particular processing conditions used and shelf storage. Examples of minerals, vitamins and other nutrients optionally present in the composition of the invention include vitamin A, vitamin $B_6$ vitamin $B_{12}$, vitamin E, vitamin K, vitamin C, folic acid, thiamine, inositol, riboflavin, niacin, biotin, pantothenic acid, choline, calcium

phosphorus, iodine, iron, magnesium, copper, zinc, manganese, chloride, potassium, sodium, selenium, chromium, molybdenum, taurine, and L-carnitine. Minerals are usually added in salt form. In addition to compatibility and stability considerations, the presence and amounts of specific minerals and other vitamins will vary somewhat depending on the intended infant population.

The complete nutritional formulas also typically contain emulsifiers and stabilizers such as soy lecithin, carrageenan, and the like. They may also optionally contain other substances which may have a beneficial effect such as lactoferrin, immunoglobulins, and the like. The complete nutritional formula can be in concentrate liquid form, liquid ready to consume form, or powder form. Of course, if in powder form, the formula is diluted to normal strength with water to be in a form ready to consume. The osmolality of the liquid nutritionally complete formula (when ready to consume) is typically about 100 to 500 mOsm/kg $H_2O$ more typically about 200 to 400 mOsm/kg $H_2O$.

According to the present invention for purposes of matching the nucleotides in the formula to the levels of the corresponding nucleotides present in human milk, only free bioavailable nucleotides are considered. The reason for following this approach is that it is believed that potentially available nucleotides which are present, for example, in polymeric forms in human milk are not broken down in time to be utilized. Additionally, it is important to match the <u>total</u> levels of each free bioavailable nucleotide to the level of the corresponding free bioavailable nucleotide present in human milk. The total levels of free nucleotides in the formula include the levels in the standard ingredients, such as, in the whey protein and the added nucleotides that are not destroyed during processing. The amounts of background nucleotide equivalents have been found to vary significantly depending on the ingredients in the formula and particularly depending on the source of protein. The background levels can range from none to significant levels. For example, standard nutritional liquid formulas based on intact cow milk proteins generally contain the following ranges of free bioavailable nucleotide equivalents:

| Nucleotide | Concentration mg/L |
|---|---|
| UMP | 3-6 |
| GMP | trace - 1.1 |
| AMP | trace - 0.3 |
| CMP | 1-4 |

The nucleotides are preferably added in form of monophosphates or salts of monophosphates. However, the added AMP nucleotide is intended to match the combined levels of free AMP, ADP, ATP and adenosine detected in human milk. The added GMP nucleotide is intended to match the combined levels of free GMP, GDP, GTP and guanosine detected in human milk. The added CMP nucleotide is intended to match the combined levels of free CMP, CDP, CTP and cytidine. Lastly, the added UMP nucleotide is intended to match the combined levels of UMP, UDP, UTP and uridine. Because recent studies indicate that free inosine nucleotides are not present in human milk, they are not currently added to the formulas.

If the levels of the background free bioavailable nucleotide equivalents vary, the amount of nucleotide equivalents that are added during the process should be adjusted to maintain the free nucleotide equivalent levels at the preferred target concentrations such as: AMP 5.0 mg/L ; GMP 4.0 mg/L; CMP 11.0 mg/L and UMP 6.0 mg/L. The total amounts of the added and the background nucleotide equivalents is generally slightly higher than the preferred target concentrations to account for losses of nucleotide equivalents during processing.

The present invention can be used in connection with any nutritional formula, including milk-based formulas and non-milk-based formulas. However, the present invention is particularly useful in connection with liquid or powder infant formulas with or without iron. Infant formulas typically include (without limitation) proteins in form of reduced minerals whey, casein, non-fat milk, vegetable proteins and casein hydrolysates. They also include vegetable oil (preferably in form of palm olein, soy, coconut and high oleic sunflower), carbohydrates (preferably in form of lactose, glucose, sucrose, starch or hydrolyzed starch), vitamins, minerals, taurine, carnitine, inositol and choline.

A typical liquid infant formula has 20 Cal per fluid ounce of the formula. However, some infant formulas have 13 Cal per fluid ounce or 24 Cal per fluid ounce. The present invention is applicable to all of these formulas. Conversion from per liter basis to calories per fluid ounce basis is within the skill of one experienced in the art of preparing nutritional formulas. Generally, processes for making nutritional formulas includes a step of heating the formula to temperature of about 200°F to 300°F, preferably 260°F to 280°F. Such processes also generally include a step of homogenizing the ingredients in the formula. In the process of the present invention, the nucleotides are preferably added to a liquid formula after the heat treatment, but before the homogenization of the formula. The nucleotides are preferably added in a pre-mixed liquid ("a premix"). A premix generally includes the amounts of nucleotides needed to produce the required concentrations in the final product and a carrier liquid. The premix can also include trace elements, such as zinc, copper and manganese.

A common concern in monitoring the well-being of infants is whether the infant is constipated. Frequency in stools is desirable without reaching the point of diarrhea. Unexpectedly, it has been found that adding free bioavailable nucleotides in amounts substantially matching levels of free bioavailable nucleotides present in human milk provides a method of reducing incidence of constipation in infants fed such formula.

The invention, therefore, provides a method of reducing incidence of constipation in infants fed the infant formulas described herein. Related to this, also provided is a method of softening stools of infant formula fed infants wherein said infants are fed a nutritional formula as described herein.

Adjustments to preparing formula as a liquid or as a dry (or powder) composition is within knowledge of those skilled in the art. For powder forms, preferably the nucleotides are dry blended with the remaining ingredients at the end of the manufacturing process.

The following examples are provided to further illustrate the invention. These examples are not intended to limit the scope of the claimed invention in any manner.

## EXAMPLE 1

In this example, the reported reactions of infants (8418 reports) to a standard commercial milk-based liquid formula (formula) was compared with the reported reactions (987 reports) of infants to the same formula which was supplemented with nucleotides (supplemented formula) such that the supplemented formula contained free bioavailable nucleotides at the following mean levels:

| | Mean (mg/L) |
|---|---|
| AMP | 3.9 |
| GMP | 2.9 |
| CMP | 9.3 |
| UMP | 6.6 |
| Total | 22.7 |

The following reactions of the infants who drank the formulas were reported:

| Reported Reaction | Formula | Supplemented Formula |
|---|---|---|
| Constipation | 14% | 4% |
| Diarrhea | 11 | 21 |
| Frequent Stool | 1 | 3 |
| | | |
| Crying | 8 | 4 |
| Fussy | 15 | 8 |
| Gas | 29 | 21 |
| | | |
| Rash | 7 | 11 |
| Refused | 6 | 3 |
| Screaming | 2 | 3 |
| Spitting Up | 20 | 24 |
| Vomiting | 16 | 16 |

The following results indicate that the nucleotides added to a milk-based formula to produce a supplemented formula having free bioavailable nucleotides at the levels specified above significantly reduced the reported incidence of constipation. The decrease in reported incidence constipation was accompanied by an increase in reports of frequent stool and diarrhea. As a result, the overall incidence of crying, fussiness and gas was decreased. The incidence of screaming and spitting up was increased slightly. The

reported rate of vomiting remained the same. The incidence of rash increased but the incidence of refusal of formula decreased.

## EXAMPLE 2

The background levels of nucleotides in a standard commercial nutritional milk-based liquid formula were determined and the formula was then supplemented with the following nucleotides (in mg/L of the formula):

| Nucleotide | Target Level (background+added) | Background Range | Added |
|---|---|---|---|
| CMP | 11.4 | 2.2 to 3.8 | 8 |
| UMP | 6.4 | 3.1 to 4.7 | 2.6 |
| AMP | 5.2 | 0.2 to 0.7 | 4.65 |
| GMP | 3 | 0.4 to 1.4 | 1.85 |
| | | | |
| Total | 26 | about 9 | 17.1 |

## EXAMPLE 3

The background levels of nucleotides in a standard commercial nutritional milk-based formula were measured and the formula was then supplemented with the following amounts of nucleotides to produce the following levels in the supplemented formula (in mg/L of formula):

| Nucleotide | Target Level (background+added) | Background Range | Added |
|---|---|---|---|
| CMP | 11.4 | 4.7 to 6.2 | 6.3 |
| UMP | 6.4 | 6.0 to 8.0 | 0.2 |
| AMP | 5.2 | 0.3 to 0.7 | 4.65 |
| GMP | 3 | 0.2 to 0.4 | 2.6 |
| | | | |
| Total | 26 | about 13 | 13.75 |

## EXAMPLE 4

A standard 20 cal/fl. oz. milk-based liquid formula was supplemented with 0.696 kg of nucleotide premix per 10,000 liters of the liquid formula. The premix contained the following amounts of nucleotides per gram of premix:

| | Mean Values |
|---|---|
| AMP | 68.17 mg |
| CMP | 84.18 mg |
| GMP | 44.00 mg |
| UMP | 8.63 mg |

## EXAMPLE 5

A standard 20 cal/fl. oz. milk-based commercial liquid formula with Iron was supplemented with 0.696 kg the liquid nucleotide premix specified in Example 4 per 10,000 L of the formula.

## EXAMPLE 6

A standard milk-based nutritional liquid concentrate was supplemented with 1.391 kg of the premix having the composition specified in Example 4 per 10,000 L of the concentrate.

## EXAMPLE 7

A standard milk-based liquid concentrate with Iron was supplemented with 1.391 kg of the premix specified in Example 4 per 10,000 L of the concentrate.

## EXAMPLE 8

A standard commercial nutritional powder formula with iron was supplemented with a nucleotide premix by dry blending the nucleotides with other ingredients of the powder formula as the last step of the process. The premix contained the following amounts of nucleotides per 100 kg of the supplemented formula:

| | |
|---|---|
| Cytidine Monophosphate | 6.510 g |
| Adenosine Monophosphate | 3.825 g |
| Disodium Guanosine Monophosphate | 3.301 g |
| Disodium Uridine Monophosphate | 3.078 g |

* * * *

Many changes and modifications of the present invention will become apparent to those skilled in the art upon studying this specification. All such changes and modifications that are within the spirit of this invention as defined by the claims are intended to be included within the scope of the claims.

**WHAT IS CLAIMED IS:**

1. A nutritional formula comprising:

(a) at least one source of non-human protein;

(b) minerals, and

(c) free bioavailable nucleotides, the concentration of said free bioavailable nucleotides matching the concentration of the corresponding free bioavailable nucleotides present in human milk.

2. The formula claimed in claim 1 wherein said source of protein comprises milk proteins.

3. The formula claimed in claim 1 wherein said source of protein comprises non-milk proteins.

4. The formula claimed in claim 2 wherein said source of protein comprises at least one of reduced minerals whey, casein, nonfat milk, and lactose-free protein.

5. The formula claimed in claim 3 further comprising at least one of lactose, a vegetable oil and taurine.

6. The formula claimed in claim 1, wherein said nucleotides include UMP, GMP, AMP, and CMP.

7. The formula claimed in claim 1, wherein the concentration of free bioavailable UMP is in the range from about 5 mg/L to about 7 mg/L of said formula, the concentration of GMP is in the range from about 3 to about 5 mg/L of said formula, the concentration of AMP is in the range from about 4 to about 6 mg/L of said formula, and CMP is in the range from about 10 to about 11 mg/L of said formula.

8. The nutritional formula of claim 1, wherein the concentration of uridine monophosphates is about 6 mg/L of said formula, the concentration of guanosine monophosphate is about 4 mg/L of said formula, the concentration of adenosine monophosphate is about 5 mg/L of said formula and the concentration of cytidine monophosphate is about 11 mg/L of said formula.

9. The nutritional formula of claim 1 wherein free bioavailable nucleotides comprise from about 3 mg/L to about 7 mg/L of AMP, from about 2 mg/L to about 6

mg/L of GMP, from about 8 mg/L to about 15 mg/L of CMP, from about 5 mg/L to about 11 mg/L of UMP.

10. The nutritional formula of claim 1 wherein the total free bioavailable nucleotides comprise mean levels of about 3.9 mg/L of AMP, 2.9 mg/L of GMP; 9.3 mg/L CMP; and 6.6 mg/L of UMP.

11. The nutritional formula of claim 1 further comprising fat, carbohydrates and vitamins.

12. In a process for the preparation a nutritional formula, which process includes the steps of heating the ingredients to a temperature in the range from about 200°F to about 300°F and a subsequent step of homogenizing the ingredients, the improvement comprising the steps of:

adding free bioavailable nucleotides to said formula after the heating step but before the homogenizing step, the type and the amounts said nucleotides matching the types of nucleotides and their levels in human milk.

13. An infant nutritional formula comprising:

(a) at least one non-human milk protein;

(b) minerals; and

(c) the following free bioavailable nucleotides:

(1) from about 5 mg/L to about 7 mg/L of UMP;

(2) from about 3 mg/L to about 5 mg/L of GMP;

(3) from about 4 mg/L to about 6 mg/L of AMP; and,

(4) from about 10 mg/L to about 11 mg/L of CMP.

14. An infant nutritional formula comprising:

(a) at least one non-human milk protein;

(b) minerals; and

(c) the following free bioavailable nucleotides:

(1) from about 5 mg/L to about 11 mg/L of UMP;

(2) from about 2 mg/L to about 6 mg/L of GMP;

(3) from about 3 mg/L to about 7 mg/L of AMP; and,

(4) from about 8 mg/L to about 15 mg/L of CMP.

15. An infant nutritional formula comprising:

(a) at least one non-human milk protein;

(b) minerals; and

(c) the following free bioavailable nucleotides:

(1) about 6 mg/L UMP;

(2) about 4 mg/L GMP;

(3) about 5 mg/L AMP; and,

(4) about 11 gm/L CMP.

16. A powder formula comprising:

(a) a source of non-human protein;

(b) minerals; and

(c) the following amounts of free bioavailable nucleotides per gram of the powder formula

(1) from about 0.039 mg to about 0.086 mg UMP;

(2) from about 0.016 mg to about 0.047 mg GMP;

(3) from about 0.023 mg to about 0.055 mg AMP;

(4) from about 0.063 mg to about 0.117 mg CMP.

17. A nutritional formula comprising free bioavailable nucleotides, the concentration of said free bioavailable nucleotides substantially matching the concentrations of the corresponding free bioavailable nucleotides present in human milk.

18. A nutritional formula according to claim 17 wherein said formula further comprises an animal derived protein and said nutritional formula is an infant formula.

19. A nutritional formula according to claim 18 wherein said formula is cow's milk based.

20. A nutritional formula according to claim 18 wherein said free bioavailable nucleotides are present in the following ranges:

(a) from about 5 mg/L to about 11 mg/L of UMP;

(b) from about 2 mg/L to about 6 mg/L of GMP;

(c) from about 3 mg/L to about 7 mg/L of AMP; and

(d) from about 8 mg/L to about 15 mg/L of CMP.

21. A nutritional formula according to claim 20 wherein said protein is in a range per 100 Cal. of total formula from about 1.8 g to about 4.5 g.

22. A nutritional formula according to claim 21 wherein said protein source comprises cow's milk intact protein.

23. A nutritional formula according to claim 22 wherein said free bioavailable nucleotides are present in the following ranges:

(a) from about 5 mg/L to about 7 mg/L of UMP;

(b) from about 3 mg/L to about 5 mg/L of GMP;

(c) from about 4 mg/L to about 6 mg/L of AMP; and

(d) from about 10 mg/L to about 11 mg/L of CMP.

24. A nutritional formula according to claim 23 wherein 5.2 mg/L AMP; 3.0 mg/L GMP; 8.0 mg/L CMP; and 2.6 mg/L UMP were added to the formula to bring the free bioavailable nucleotide levels within said ranges (a) - (d).

25. A nutritional formula according to claim 23 wherein 4.65 mg/L AMP; 2.6 mg/L GMP; 6.3 mg/L CMP; and 0.2 mg/L UMP were added to the formula to bring the free bioavailable nucleotide levels within said ranges (a) - (d).

26. A nutritional formula for infants according to claim 17 wherein said formula further comprises a vegetable derived protein and said nutritional formula is for infants.

27. A nutritional formula according to claim 26 wherein said free bioavailable nucleotides are present in the following ranges:

(a) from about 5 mg/L to about 11 mg/L of UMP;

(b) from about 2 mg/L to about 6 mg/L of GMP;

(c) from about 3 mg/L to about 7 mg/L of AMP; and

(d) from about 8 mg/L to about 15 mg/L of CMP.

28. A nutritional formula according to claim 27 wherein said protein is in a range per 100 Cal. of total formula from about 1.8 g to about 4.5 g and said vegetable of said vegetable based protein is soy.

29. A nutritional formula according to claim 28 wherein said free bioavailable nucleotides are present in the following ranges:

(a) from about 5 mg/L to about 7 mg/L of UMP;

(b) from about 3 mg/L to about 5 mg/L of GMP;

(c) from about 4 mg/L to about 6 mg/L of AMP; and

(d) from about 10 mg/L to about 11 mg/L of CMP.

30. A nutritional formula according to claim 29 wherein said formula further comprises a carbohydrate, a lipid and minerals and vitamins to make the formula sufficiently nutritionally complete for said infant.

32. A method for softening stools in infants comprising feeding the formula of claim 20 to said infant.

33. A method of reducing the incidence of constipation in an infant comprising feeding the formula of claim 20 to said infant.

34. A method for softening stools in infants comprising feeding the formula of claim 23 to said infant.

35. A method of reducing the incidence of constipation in an infant comprising feeding the formula of claim 23 to said infant.

36. A method for softening stools in infants comprising feeding the formula of claim 29 to said infant.

37. A method of reducing the incidence of constipation in an infant comprising feeding the formula of claim 29 to said infant.

38. A method for softening stools in infant formula fed infants comprising:

(a) supplementing the formula with nucleotides such that the levels of free bioavailable nucleotides substantially matches the concentrations of the corresponding free bioavailable nucleotides in human milk; and

(b) feeding the formula of step (a) to an infant.

39. A method for reducing the incidence of constipation in infant formula fed infants, said method comprising the following steps:

(a) supplementing the formula with nucleotides such that the levels of free bioavailable nucleotides substantially matches the concentrations of the corresponding free bioavailable nucleotides in human milk; and

(b) feeding the formula of step (a) to an infant.

40. In an improved process for making dry nutritional powder, the improvement comprising:

(a) blending the following amounts of free bioavailable nucleotides to produce a pre-mix:

(1) 6.510 g CMP;

(2) 3.825 g AMP;

(3) 3.301 g GMP;

(4) 3.078 g UMP.

(b) dry blending the amount of premix produced in step (a) per 100 Kg of other ingredients of the dry nutritional powder.

41. A process for producing supplemented formula, said process comprising the following steps:

(a) determining the background levels of free bioavailable nucleotides, including UMP, GMP, AMP and CMP in the formula;

(b) adding sufficient amounts of each free bioavailable nucleotide to substantially match the total concentration of each nucleotide to the concentration of the corresponding free bioavailable nucleotide in human milk.

42. A process for preparing infant formula comprising the steps of:

a) measuring a protein source in said infant formula for background levels of free bioavailable nucleotides present in said protein source;

b) supplementing said formula with free bioavailable nucleotides to provide said formula with concentrations of free bioavailable nucleotides that substantially match the mean levels of nucleotides found in human milk.

43. A process for preparing infant formula according to claim 42 wherein said formula comprises said protein source, one or more sources of carbohydrates, one or more sources of lipids, and vitamins and minerals.

44. A process for preparing infant formula according to claim 43 wherein said concentrations of free bioavailable nucleotides are present in said infant formula in the following ranges:

(a) from about 5 mg/L to about 11 mg/L of UMP;

(b) from about 2 mg/L to about 6 mg/L of GMP;

(c) from about 3 mg/L to about 7 mg/L of AMP; and

(d) from about 8 mg/L to about 15 mg/L of CMP.

45. A process for preparing infant formula according to claim 42 wherein said concentrations of free bioavailable nucleotides are present in said infant formula in the following ranges:

(a) from about 5 mg/L to about 7 mg/L of UMP;

(b) from about 3 mg/L to about 5 mg/L of GMP;

(c) from about 4 mg/L to about 6 mg/L of AMP; and

(d) from about 10 mg/L to about 11 mg/L of CMP.

46. A process according to claim 42 wherein the amounts of the free bioavailable nucleotides used to supplement said formula are 3.9 mg/L AMP; 2.9 mg/L GMP; 9.3 mg/L CMP; and 6.6 mg/L UMP.

47. A process according to claim 42 wherein the amounts of the free bioavailable nucleotides used to supplement said formula are 3.9 mg/L AMP; 2.9 mg/L GMP; 8 mg/L CMP; and 2.6 mg/L UMP

# INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 97/14111

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/30 A23C9/152 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A23C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | JANAS L.M; PICCIANO M.F.: "THE NUCLEOTIDE PROFILE OF HUMAN MILK" PEDRIATIC RESEARCH, vol. 16, no. 8, 1982, ILLINOIS,USA, pages 659-662, XP002048454 cited in the application --- | 1-30, 40-47 |
| A | LEACH J.L.; ET AL: "TOTAL POTENTIALLY AVAILABLE NUCLEOSIDES OF HUMAN MILK BY STAGE OF LACTATION" AMERICAN JOURNAL OF CLINICAL NUTRITION, vol. 61, no. 6, 1995, USA, pages 1224-1230, XP002048455 cited in the application --- -/-- | 1-30, 40-47 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 3 December 1997 | 09. 01. 98 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Caturla Vicente, V |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 97/14111

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DE 12 14 075 B (TAKEDA CHEMICAL INDUSTRIES,LTD.) 7 April 1966<br>see line 51-68; claim 5 | 7-10, 13-16, 20, 22-25, 27,29,30 |
| A | EP 0 302 807 A (UNION INDUSTRIAL Y AGROGANADERA S.A.- UNIASA) 8 February 1989<br>& US 5 066 500 A<br>cited in the application | 7-10 |
| A | WO 95 18618 A (ABBOTT LABORATORIES) 13 July 1995<br>& US 5 488 039 A<br>cited in the application | 7-10, 13-16, 20, 22-25, 27,29, 40-47 |
| A | US 3 231 385 A (ZIRO; ET AL) 25 January 1966<br>cited in the application | 7-10 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int. .ational application No.
PCT/US 97/14111

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 1-6,17-19,26

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

lack of conciseness of claim 1,17 and dependent claims; the specific concentrations of the nucleotides are needed for a meaninful search .Specially the wording "substantially matching the concentration" is too vague.

Remark : Although claims 32-39
are directed to a method of treatment of
the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat l Application No
PCT/US 97/14111

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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